Human Tau [pT181]Singleplex Bead Kit

Technical Data Sheet

Catalog #: LHB7051 **Lot #:*** 846176

*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

Intended Use

This kit comprises of components for the measurement of human Tau when phosphorylated at threonine 181 in CSF and tissue culture media. These reagents are also suitable for the measurement of Tau [pT181] in cell lysates. Buffer reagents needed to complete the reaction are sold separately under Catalog # LNB0001 (Invitrogen's Neuroscience Buffer Reagent Kit). These reagents are intended for use in the **Luminex® 100™ or 200™ System** only. **This kit is configured for research use only and is not to be used in diagnostic procedures.**

Reagents Provided

Hu Tau [pT181]

1. **Part #:** LM144 **Description:** Antibody Bead Concentrate (10X) **Lot:** 846180 **Size:** 0.25 mL 100 tests

Bead Region: 69

Form: 0.25 mL 10x bead concentrate solution in storage buffer. Contains 0.05% sodium azide as a preservative.

Storage: Store at 2 - 8°C until the expiration date indicated on the kit.

Recommended Dilution: Use 25 µL of diluted bead solution per assay. For a single analyte assay, mix 1 part 10x bead concentrate with 9 parts Working Wash Solution. To develop a multiplexed assay that includes other Neuroscience Singleplex Bead Kits, mix equal volumes of the different 10x bead concentrates, then dilute the beads with Working Wash Solution to yield a 1x bead mixture. For example, to develop a 3-plex assay, mix equal parts of each of the three bead concentrates and dilute with 7 parts Working Wash Solution. See the User Manual included in the Neuroscience Buffer Reagent Kit for further information.

Note: Beads for Human Total Tau, Tau [pT181], and Tau [pS199] cannot be mixed. Each of these analytes must be measured separately.

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2. **Part #:** DN144 **Description:** Detection Ab Concentrate (10x) **Lot:** 846178 **Size:** 0.5 mL-100 tests

Form: 0.5 mL of a 10x stock of Detector Antibody Concentrate in Detector Antibody Diluent. Contains 0.1% Sodium Azide as a preservative. Concentration of antibody is matched to this lot of beads. Do not mix lots of Coated Beads and Detector Antibody.

Storage: Store at 2 - 8°C until the expiration date indicated on the kit.

Recommended Dilution: Use 50 µL of diluted Detector Antibody solution per assay. For a single analyte assay, mix 1 part 10x Detector Antibody concentrate with 9 parts Detector Antibody Diluent. To develop a multiplexed assay that includes other Neuroscience Singleplex Bead Kits, mix equal volumes of the different 10x Detector Antibody Concentrates, then dilute the antibodies with Detector Antibody Diluent to yield a 1x Detector Antibody mixture. For example, to develop a 3-plex assay, mix equal parts of each of the three Detector Antibody Concentrates and dilute with 7 parts Detector Antibody Diluent. See the Product Insert included in the Neuroscience Buffer Reagent Kit for further information.

3. **Part #:** SM055 **Description:** Hu Tau [pT181] Standard **Lot:** R090608 **Size:** 2 Vials

Form: This Tau [pT181] standard (lyophilized recombinant protein) is designated in pg/mL. The protein in this standard has been calibrated with the respective Invitrogen phosphoELISA™ kit. Detailed information on calibration is provided on the accompanying page. Contains 0.05% sodium azide as a preservative.

Storage: Store at 2 - 8°C. Use within 1 hour after reconstitution. Discard immediately after use.

Concentration of Reconstituted Standard: Tau [pT181] 30,000 pg/mL**

****Important note: The concentration of reconstituted standard is lot-specific. Please verify all concentration values entered in data analysis software.**

Reconstitution: When working with CSF samples and cell lysate samples, reconstitute in 0.5 mL Assay Diluent. When working with tissue culture medium samples, reconstitute with 0.5 mL of a mixture of 50% Assay Diluent and 50% tissue culture medium.

Recommendations for Combining with Other Standards: When combining with one other standard, reconstitute with 0.5 mL. Combine equal volumes of the two standards. When combining with two other standards, reconstitute with 0.333 mL, and combine equal volumes of the three standards. Up to four standards may be combined.

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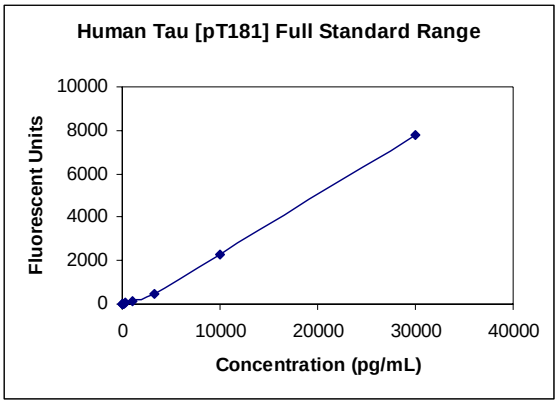
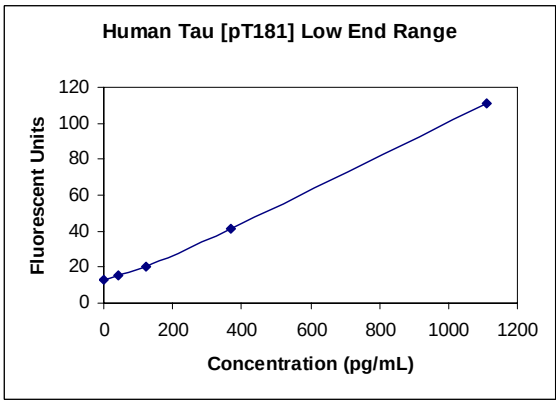
Recommended Starting Concentration for Standard Curve: Upon reconstitution, the starting concentration of standard is the value cited above. Make serial 1:3 dilutions in Assay Diluent (CSF and cell lysate samples) or mixture of 50% Assay Diluent and 50% tissue culture medium (tissue culture medium samples). Each well designated for standard receives 50 µL of 1x Detector Antibody followed by 50 µL standard. Each well designated for sample receives 50 µL of 1x Detector Antibody followed by 25 µL Assay Diluent and 25 µL sample. See the Product Insert included in the Neuroscience Buffer Reagent Kit for further information.

Performance Characteristics

Analytical Sensitivity: The analytical sensitivity of the Tau [pT181] assay is <50 pg/mL. This was determined by adding two standard deviations to the mean of the fluorescence intensity units obtained when the zero standard was assayed 24 times.





Representative Standard Curve

Species Reactivity: The Human Tau [pT181] Singleplex Bead Kit is specific for human Tau when phosphorylated at threonine 181. It does not cross-react with rat or mouse. Other species were not tested.

Specificity: Buffered solutions of a panel of substances with concentrations ranging between 1 and 35 ng/mL were assayed with the Invitrogen Human Tau [pT181] Singleplex Bead Kit. The following substances were tested and found to have no cross-reactivity: Hu Aβ40, Aβ42, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFN-α, IFN-γ, GM-CSF, TNF-α, IL-12, IL-13, IL-15, IL-17, MCP-1, MIP-1α, MIP-1β, Eotaxin, RANTES, IP-10, MIG, EGF, FGF basic, G-CSF, HGF, VEGF, DR5, IL-1RA, IL-2R, IL-4R, sTNF-RI, sTNF-RII, GRO-α, MCP-2, and MCP-3. This kit does not cross-react with non-phosphorylated Tau.

Precision:

	Intra-assay (n=16)	**Inter-assay** (n=32)
Mean (pg/mL)	12081.10	12151.30
SD	195.42	217.36
%CV	1.62	1.79

Linearity of Dilution: CSF and tissue culture medium (DMEM containing 10% FBS) were spiked with human Tau [pT181] and serially diluted in Assay Diluent or 50/50 Assay Diluent/tissue culture medium over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 for CSF and a correlation coefficient of 0.99 for tissue culture medium.

Recovery:

CSF averaged 122%. Tissue culture medium containing 10% FBS averaged 97.5%.

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Correlation to ELISA:
A correlation coefficient of 0.99 was calculated when values for natural Tau [pT181], obtained with the Hu Tau [pT181] Singleplex Bead Kit, were compared with the Invitrogen ELISA for Hu Tau [pT181]. With natural sample, Hu Tau [pT181] Singleplex Bead Kit (pg/mL) x 0.73 = Hu Tau [pT181] ELISA (pg/mL). Fifty picograms measured with this Hu Tau [pT181] Singleplex Bead Kit are equal to 1 Unit, as determined by the Invitrogen Hu Tau [pT181] ELISA kit. Correlation of results obtained with the Hu Tau [pT181] Singleplex Bead Kit to one's own system should be determined to arrive at an appropriate multiplication factor.

Cell Lysate Sample Preparation:
In addition to CSF and tissue culture medium samples, this kit has been used to detect Hu Tau [pT181] from lysates made from SH-SY5Y cells, prepared in Cell Lysis Buffer (Invitrogen Cat. # FNN0011: 10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate, 1 mM PMSF [added fresh from 0.1 M stock in DMSO], and protease inhibitor cocktail [added fresh from stock made using Sigma Cat. # P-2714]) and diluted at least ten-fold in Assay Diluent. To produce a lysate, incubate cells with cell lysis buffer (1-2 x 10^8 cells/mL is recommended) on ice for 30 minutes, vortexing at 10 minute intervals, then clarify the lysate by centrifugation at 13,000 RPM for 10 minutes. Cell lysates may be stored at -80°C for up to three months with one freeze/thaw cycle. Optimization of cell stimulation and cell lysis procedures may be required for each specific application.

Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

By purchasing this Kit, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ("Luminex®"), you, the customer, acquire the right under Luminex's patent rights to use this Kit or any portion of this Kit, including without limitation the microsphere beads contained herein, only with Luminex's laser based fluorescent analytical test instrumentation marketed under the name Luminex® 100™ or 200™ System. This product is covered by one or more of the following U.S. patents: 6,046,807

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